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LabLink

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Bureau of Laboratories

Vol. 7 No. 2 **Winter 2002**

Reflections on the Times

Frances Pouch Downes, Dr. P.H.
Laboratory Director

Since the tragic events of September 11…. How many articles, pleas and speeches lately have started this way? Yet these words have not yet lost their poignancy. They are not cliché. The words convey the understanding that our personal and professional lives are different today than they were a few months ago: air travel changes, increased security in our workplaces, government offices and schools and bioterrorism. The threat of bioterrorism has focused national attention to public health laboratories.

It's not if, it's when… these words were once used to draw attention to the risk of a bioterrorist attack. When the theoretic threat became real, the public health laboratory system responded. The Michigan Department of Community Health Laboratory has tested over 300 environmental samples for the presences of *Bacillus anthracis*. All results have been negative as of mid-December. Nationwide public health laboratories have tested thousands of these samples. During this time of intensive and urgent testing, public health laboratories have not been overwhelmed. We are working hard and for many hours to keep up with testing samples determined by law enforcement to represent credible threats. Public health laboratory professionals have shown personal and professional commitment on par with any first responder. I am so proud and privileged to be associated with this group of professionals.

Public health laboratories have not been alone in the on-going laboratory response to the bioterrorism threat in Michigan. Recognizing the increase in workload, partners in other state government, university and clinical laboratories offered to lend assistance. Special recognition goes to University of Michigan Hospital and the Michigan Department of Agriculture. Thanks to others who offered assistance. Testing clinical specimens and early recognition of illness depends on well-trained clinical laboratories. In Michigan many laboratories were better prepared to face the crisis due to participating in MDCH-sponsored training. The training also paved the way for development of rapid communication tools to laboratories and hospitals. The laboratory communications system continues to be used as urgent information needs to be disseminated to clinical practice.

While the preparation taken for an adequate response to the initial threats was considerable, we are not done preparing. Please consider the following points in determining your level of preparedness:

Training: If your microbiology laboratory has not yet received training to recognize agents of bioterrorism in clinical specimens, contact MDCH for clinical laboratory in-service training. If your have received this training, review the materials provided in training and those provided by the Centers for Disease Control.

Communications: If your laboratory is not receiving urgent faxes from MDCH, contact the Bureau of Laboratories to enroll in the rapid communication system.

Build collaborations: To ensure a strong system of integrated public health and clinical laboratories, the CDC has initiated the development of a national system. The current threat demonstrates that this system is needed to detect and respond to health threats (see page 2). Throughout the next year a series of local opportunities to link public health and clinical laboratories will be initiated.

Bug Bytes

John Dyke, Ph.D.
Bureau of Laboratories

No, this is not a spelling error. A byte is a small piece of information. This is a new column to the *LabLink* newsletter. This issue will introduce a new national initiative that the Bureau of Laboratories is undertaking in cooperation with the Centers for Disease Control and Prevention. The program is called the National Laboratory System Demonstration Project. The major focus is to ensure a strong integrated system of public health, hospitals and independent laboratories. The goal is to improve protection of the public's health.

Collaborative communication is the key for success in rapid identification through better and proven laboratory procedures that address agents of public health importance. All microbiology laboratories need to be able to detect and respond specifically to acute threats such as bioterrorism or outbreaks of *E. coli* H7:0157, as well as chronic public health threats, such as development of antimicrobial resistance.

The intent of this column is to keep laboratories informed and involved in this program. By now most labs should have had or are scheduled for the on-site training on methods for recovering agents of bioterrorism. The procedures that are provided as part of this training are invaluable. If your facility has not taken advantage of this training, please contact MDCH at 517-335-8183 to be scheduled.

By now, all laboratories should have received a fax mail questionnaire on *E. coli* H7:0157. Please take a few minutes to answer the questions and return it by the fax number provided on the form. The results of the survey will be shared with all laboratories. The name of the responders is confidential and will not be released. Input on other issues to accomplish this goal will be solicited in the near future.

Upcoming issues of the *LabLink* will deal with other topics important to microbiologists in their attempt to keep current with the rapidly changing environment of public health.

Lead Hurts Kids

Jeff Dupler
Trace Metals Section

With the onset of winter, children will be spending more and more time indoors. Playing inside the home, daycare facility or preschool can be dangerous for children if lead hazards are lurking, especially for young toddlers who seem to put everything into their mouths.

Children under the age of six years are busy developing their brain and nervous system. Children are at higher risk during this developmental period for damage by lead dust that may be present in their environment. A myth exists that children must be eating paint chips to become lead poisoned. In fact, it is pre-1978 paint that has crumbled into lead dust that is the culprit. There are some simple things that can be done to prevent the lead dust from getting into the children's bodies and causing damage.

Be on the lookout for chipping and peeling paint in pre-1978 homes and facilities. Obvious paint chips in window troughs, window sills, or on the floor near windows or doors should be removed. Take a damp paper towel, wipe up the chips and dispose of the paper towels in a plastic trash bag. If possible, spray the area with water mist, wet scrape the paint, and stabilize it with two coats of latex paint. If paint stabilization is not affordable, temporarily cover the hazard with duct tape, contact paper or move a piece of heavy furniture in front of the hazard to prevent the child's access to the area. When cleaning the floor and furniture, always use a vacuum with a HEPA filter. These vacuums are available on loan from many local health departments. Call your local health department to inquire about availability.

For further information about childhood lead poisoning and ways to prevent it, watch for articles in future issues of *LabLink* or call Michigan Department of Community Health, Childhood Lead Poisoning Prevention Program, at (517) 335-8885. For assistance with remediation and abatement of lead hazards, call the Lead Hazard Remediation Program toll free at (866) 691-LEAD (5323)

Salmonella Serotyping

Carrie Anglewicz, Hao Trinh and
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Enteric - STD - Chromatography Unit

Each year MDCH enteric serotyping personnel compile a listing of the Salmonella isolates serotyped the previous year. This is shared with MDCH epidemiology personnel to be used in their work duties. In addition, a top ten frequency list of the previous five years is updated each year. The majority of *Salmonella* spp. isolates (approximately 71 percent) identified at MDCH over the past five years are on this list. There are over 2,000 possible known serotypes of *Salmonella*. Knowing the most frequently isolated serotypes allows the microbiologist to quickly screen for them and get those reports out much faster and spend additional time with the more rare, atypical or difficult to type strains.

In the last five years, there were 164 different serotypes identified after examining 5293 *Salmonella* isolates. This chart shows the Salmonella frequency of human isolates during the last five fiscal years (October 1 to September 30.)

Serotype	1997	1998	1999	2000	2001	Total	% Total
TYPHIMURIUM	303	318	320	219	196	1356	25.62
ENTERITIDIS	206	269	227	185	220	1107	20.92
HEIDELBERG	61	86	60	74	84	365	6.90
NEWPORT	20	30	42	36	50	178	3.36
THOMPSON	47	42	34	24	19	166	3.14
ORANIENBURG	20	48	31	18	19	136	2.57
JAVA	15	29	26	28	35	133	2.51
AGONA	38	52	15	10	11	126	2.38
MUENCHEN	12	23	18	43	15	111	2.10
BRAENDERUP	12	10	11	65	4	102	1.93

The following chart is for Salmonella non-human isolates over the last five years. It is the top 12 serotypes due to a three way tie for the tenth position. There were 67 different serotypes identified from 643 non-human isolates. Non-human isolates come from foods implicated in food borne disease outbreaks, pets, poultry and livestock.

Serotype	1997	1998	1999	2000	2001	Total	%Total
TYPHIMURIUM	48	50	37	12	12	159	24.73
DERBY	1	1	7	12	18	39	6.07
RISSEN	0	0	0	0	36	36	5.60
INFANTIS	0	0	2	33	0	35	5.44
THOMPSON	11	1	1	5	4	22	3.42
HEIDELBERG	8	1	0	0	12	21	3.27
UGANDA	0	0	3	8	10	21	3.27
MUENSTER	7	1	3	2	2	15	2.33
MONTEVIDEO	2	0	8	1	1	12	1.87
CERRO	2	5	0	2	2	11	1.71
DUBLIN	1	2	5	2	1	11	1.71
SENFTENBURG	7	0	0	0	4	11	1.71

Polio Virus Update

The World Health Organization reports worldwide progress toward the eradication of polio with transmission of wild virus continuing in only a few areas of the world. As wild polio transmission ceases, efforts will be directed toward preventing reintroduction of polio from clinical and research laboratories. As a first step toward preventing polio reintroduction in the post-eradication era, laboratories are encouraged to appropriately dispose of un-needed wild polio virus stock or potentially infectious materials (i.e., throat, fecal or environmental [water or sewage] specimens collected for any purpose at a time and in a geographical location where polio was endemic). Not only virology laboratories might be storing these materials. The specimens may have been collected for nutritional, parasitologic, bacteriologic, environmental or other studies. All laboratories which have long-term storage capabilities will be surveyed to establish a national inventory of laboratories retaining such materials.

MMWR. Global progress toward laboratory containment of wild polioviruses, June 2001. 50(29):620-3.

FUN FUNGI.....

Differentiating *Bipolaris* spp. from *Drechslera* spp.

Sandy Arduin & Bruce Palma - Mycobacteriology/Mycology Unit

Bipolaris spp. is often observed as a contaminant in clinical specimens but may occasionally cause infections of various sites including the eye, sinuses, bones, lung, brain and skin. The color of the colony surface first appears white to grey becoming dark olive to black with time. The color on the reverse side of the colony is typically black. Microscopically the conidiophores are septate, brown and geniculate (the conidiophore bends at the point where each conidium is formed). The conidia are brown, thick walled, and distoseptate (cells not separated by conventional septa but are contained in sacs which have walls distinct from the outer wall of the conidia). The conidia typically contain 3-6 cells and have a scarcely protuberant hilum (a conspicuous scar remaining on the base of the conidia where it separated from the conidiogenous cell).

Drechslera spp. are typically isolated from plants and soil. Some species are plant pathogens. The only species known to cause human infection is *Drechslera biseptata*. Other *Drechslera* spp. previously reported as pathogenic are now considered members of the genera *Exserohilum* and *Bipolaris.* The color of the colony surface first appears white, becoming olive brown to black with time. The color on the reverse side of the colony is typically black. Microscopically the conidiophores are septate, brown and geniculate. The conidia are brown, distoseptate, contain 3-6 cells and are without a protuberant hilum.

Differentiating *Drechslera* spp. from *Bipolaris* spp:

Bipolaris spp. produce conidia profusely. The conidia have an average size of 8 x 26Fm. *Drechslera* spp. produce conidia poorly. The conidia have an average size of 16 x 65Fm. These differences provide preliminary identification of the two genera. Examination of germ tube production significantly assists in determining the final differentiation of these two genera.

To perform a germ tube examination[1]:

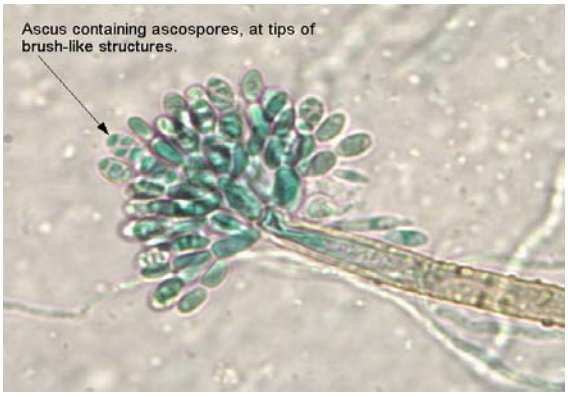
1. Place a drop of water on a sterile microscope slide.
2. Inoculate the drop of water with condia from an actively growing fungus.
3. Examine the slide microscopically to confirm that conidia are present.
4. Place a sterile coverslip over the suspension.
5. Incubate in a moist chamber at room temperature for 8 to 24 hours.
6. Examine the slide microscopically to determine the presence and structural orientation of the germ tubes.

The germ tubes of *Bipolaris* spp. originate from one or both end cells adjacent to the hilum, and grow in the direction of the long axis of the conidia. The germ tubes on *Drechslera* spp. originate from either an intermediate cell or from the end cells, but not adjacent to the hilum, and grow perpendicular to the conidial axis.

***Bipolaris* Germtube**

***Drechslera* Germtube**

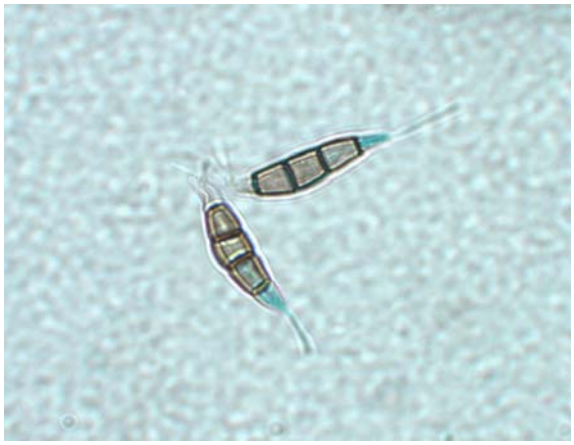
Last Issue's Picture Quiz Answer:
The photo was a picture of *Cephaloascus fragrans*.



Cephaloascus fragrans is the only member of the family Cephaloascaceae. It resembles the yeast-like fungi of the Endomycetaceae family. It differs from others in that it produces four, rarely eight, hat shaped ascospores in asci which are formed by budding at the tip of an ascophore.

This issue's picture quiz: What Mould Is This?

This isolate was received as a referred culture from a nail specimen. Growth was white and cottony; developing dark black sections with age. Microscopically, the conidia appeared brown, fusiform (spindle shaped) with three to five cells and had two setulae at each end.



[1] Larone, D.H., *Medically Important Fungi, A Guide to Identification* , Third Edition, 1995, ASM Press, Washington, D.C.

Activity of West Nile and Other Arboviruses in Michigan, 2001

Duane W. Newton, Ph.D.
Virology/Immunology Section

The summer of 2001 marked the introduction of West Nile virus (WNV) into Michigan. This was part of a major southern and western expansion of the geographic distribution of WNV in the U.S. this year. The surveillance activities for the detection of WNV in Michigan were part of a cooperative plan developed between the Michigan Department Community Health (MDCH), Michigan Department of Agriculture (MDA), Michigan Department of Natural Resources, and Michigan State University's Animal Health Diagnostic Laboratory (AHDL) and Department of Entomology. The framework of the plan (*LabLink*, Vol.6 No.4) was presented to local health departments and affiliated local agencies on several occasions during the spring. Many local agencies utilized this information, as well as additional information from federal agencies, to develop surveillance and response plans specific for their areas. The following summarizes activity in the state for this year's season.

Dead Bird Surveillance

The identification, almost simultaneously, of the first two WNV-positive crows (one each in Macomb and Oakland Counties) began a whirlwind of activity that included submission and testing of additional birds, organizing mosquito surveillance activities, and communicating information across the state. The WNV hotline that was established for the reporting of dead birds went from receiving approximately 10 to 20 calls a day before the first positives, to over 100 calls a day after. Table 1 shows a time line of submission of the first positive birds from the jurisdictions where WNV was detected this year. It became clear early on that the continued detection of positive birds from the same geographic area would not provide any additional, relevant surveillance data. The state-level multi-agency working group initially decided to discontinue accepting specimens for testing from municipalities or townships in which a WNV-positive bird had been detected. This embargo was broadened to the county level, such that no further specimens were tested from counties with a documented WNV-positive bird. Table 2 shows a summary of the specimens submitted and tested during this season. The large number of specimens that were not tested were due to a combination of specimens received in poor condition and specimens received from areas that had already been embargoed. An additional change during the season was to include bluejays as an acceptable specimen. Bluejays constituted 77 of the birds received for testing, with two found to be WNV-positive.

Mosquito Surveillance

Shortly after the identification of the first positive birds from Macomb and Oakland counties, targeted mosquito surveillance was conducted in areas consisting of two mile radius circles around the spots where these dead birds were found. Targeted collection took place over the course of four days and involved rotating collection sites within these circles. A total of 108 pools of 13 different mosquito species were collected, including those known to transmit WNV between birds (such as *Culex* spp.) as well as those suspected of being bridge vectors between birds and humans (such as *Aedes* spp.). These pools were tested for WNV by polymerase chain reaction (PCR) at MDCH. Two pools of *Culex pipiens* (one each from Macomb and Oakland counties) were found to be positive. Several hundred pools have also been collected from other counties that had WNV-positive birds. Those are in the process of being tested. These targeted mosquito collections were performed in conjunction with the ongoing mosquito surveillance activities coordinated between MDA and local health departments in the Saginaw Bay area and across the southern tier of lower Michigan. Mosquitoes collected through these efforts are routinely tested for Eastern Equine Encephalomyelitis virus (EEE) and St. Louis Encephalitis virus (SLE) by antigen capture at the MDA laboratory. This season, two pools of *Culiseta morsitans,* one each from Tuscola and Bay counties, were found to be positive for EEE. These were the only other arbovirus-positive mosquito pools detected through the statewide surveillance program.

Human Case Surveillance

As part of surveillance for cases of human arboviral disease, the MDCH Virology Section tested 153 specimens from 120 patients suspected of having arboviral encephalitis. Single serum and/or CSF specimens were tested for IgM antibodies against WN, EEE, SLE, and California group (LaCrosse Encephalitis) viruses. One case of arboviral encephalitis was detected through these efforts. This was a fatal case of EEE in a 14 year old from Livingston County (onset during first week of September). The only other EEE activity detected in the state this year were the previously mentioned mosquito pool (collected the last week of August) and a group of live-captured birds from Kalamazoo County that demonstrated antibodies against EEE (sera collected June-July).

Equine Case Surveillance

MDA worked closely with veterinarians and horse owners around the state in performing active surveillance for suspected equine cases of arboviral encephalitis. In spite of this, no horse specimens were submitted to AHDL for testing. MDA will continue to work very closely with equine owners and veterinarians during next year's season as the recently released equine vaccine against WNV will likely be available in Michigan.

Challenges for 2002

While the surveillance efforts were successful in detecting the introduction and tracking the movement of WNV in Michigan during 2001, there were several areas that can be improved to make the system one that is more effective and efficient. These include having a stock of shipping kits in place ahead of time to deal with the rush of requests during the outbreak; developing more timely reporting of test results to submitting agencies; devising a plan for mosquito surveillance that provides accurate and useful data without overwhelming specimen collection and testing systems. Any additional comments, suggestions, or opinions are welcome. Contact Dr. Duane Newton in the Bureau of Laboratories at (517) 335-8099 or Dr. Mary Grace Stobierski in the Bureau of Epidemiology at (517) 335-8165.

Table 1. Submission date for first WNV-positive bird, by jurisdiction.

Jurisdiction	Submission date
Macomb Co.	08 Aug 2001
Oakland Co.	10 Aug 2001
City of Detroit	21 Aug 2001
Washtenaw Co.	24 Aug 2001
Ingham Co.	27 Aug 2001
Jackson Co.	27 Aug 2001
Wayne Co.	29 Aug 2001
Barry Co.	07 Sep 2001
Calhoun Co.	07 Sep 2001
Muskegon Co.	20 Sep 2001
Ottawa Co.	27 Sep 2001

Table 2. Summary of West Nile virus dead bird testing–Michigan, 2001*

Specimens submitted	581
Specimens tested	240
Specimens positive	65
Jurisdictions with positives	11

*Through October, 2001

Comparison of Collection Procedures for Blood Lead Analysis

Jeff Dupler, Trace Metals Section

Childhood lead poisoning is one of the most common preventable child health problems in the United States today. The toxic affects of lead are most severe in the developing nervous systems of young children and fetuses. Many cases of lead poisoning go undetected because children suffering from lead poisoning often show no acute symptoms.

Analytical chemistry is an evolving science with new technologies emerging continually. New instruments, analytical procedures and collection techniques for lead are expected to measure <10 ug/dL. This value is presently the "acceptable" or "tolerable" level blood lead concentration. Values greater than that are considered the action level for lead toxicity according to a Centers for Disease Control and Prevention (CDC) statement in 1991[1]. New laboratory methodologies would ideally permit not only the immediate initial screening but also the retesting for confirmation of children determined to have elevated blood lead results. These rapid methods would be very useful in resource poor areas which cannot afford other laboratory-based methods, or have no infrastructure for rapid transport of blood samples to a centralized laboratory facility. As methods develop, evaluations of method performance also need to be updated.

There have been numerous studies on the collection procedures for determining blood lead levels. CDC guidelines[1] indicate that the preferred test for detecting lead effects in children is the measurement of lead in whole blood. The current collection methods commonly used are 1) venipuncture (venous tube) or 2) capillary (using micro-collection container tube [microtainer] or filter paper). The vacutainer and microtainer tube collection methods have been documented extensively with clear collection guidelines (National Committee for Clinical Laboratory Standards [NCCLS] June, 2001 approved guideline C40-A, *Analytical Procedures for the Determination of Lead in Blood and Urine; Approved Guideline).* The use of dried blood spots on filter paper for blood lead measurements (FP/Pb) is cited in this guideline. Recent investigations have reported the successful use of the method for the screening and identification of patients with blood lead levels above 10 ug/dL. However, extensive collection procedures for FP/Pb are not addressed in the C40-A NCCLS guideline. The NCCLS October, 1997 approved standard, *Blood Collection on Filter Paper for Neonatal Screening Programs-Third Edition,* addresses the specimen acceptability, source of the blood, paper, ink, transport specifications, contamination and techniques for capillary blood collection on FP for newborns. A similar guideline should be developed for blood lead testing on FP. The November, 1997 CDC[2] document also defines an important difference between the uses for capillary and venous samples in lead testing. Capillary samples should be used for screening purposes only. A venous sample is necessary for confirmation of screening results and is required for diagnosis and treatment of lead toxicity.

The high potential for lead contamination during blood collection has been recognized. Blood collected by venipuncture is the best sampling technique because it limits the opportunity for specimen contamination due to the small exposure that occurs as the venipuncture needle punctures the skin surface. When metals free vacutainers are used, the venous collection has essentially negligible potential for environmental contamination. When capillary sampling is substituted for venous sampling, rigorous wound site cleansing is necessary to prevent specimen contamination in both the microtainer and FP methods. Thorough patient hand washing is a critical part of a good blood lead specimen collection technique.

Capillary collection does have potential advantages: a relatively a small amount of blood is required, blood collected via finger stick requires less technical skill and can be less traumatic to children. The use of plastic microtainers for capillary collection serves to sequester blood from environmental contamination once the collection from the finger has taken place. The microtainers can also be certified lead-free by the manufacturer or tested for lead by the laboratory. Substitution of FP collection of capillary blood introduces additional variables which may lead to false-positive rates. Problems may include nonreproducibility of blood spotting, variable content of the FP, environmental contamination with the sampling process itself when blood is dropped onto the FP and air dried. Laboratory method differences may cause low extraction recoveries of lead from the FP. The biggest advantage of FP is the ease in transporting the specimen. CDC continues to recommend that all blood collection materials be prescreened and lot tested for lead contamination regardless of which type of collection is used.

In February 1999, the CDC[3] presented an assessment of a pilot proficiency testing (PT) filter paper program. The results initially showed poor agreement with specimen target values, but significant improvement in the five laboratories participating in later events (two laboratories dropped out). The widely varied performance appeared to be testing method dependent. CDC stated that filter paper techniques are acceptable for blood lead testing if health care providers ensure that the chosen laboratory is participating satisfactorily in the CLIA certified PT program. Currently the Wisconsin State Laboratory of Hygiene offers the only FP matrix blood lead PT program. The CDC[3] also includes the statement that this position is not an endorsement of the use of filter paper over other techniques for the purpose of sample collection or analysis for blood lead.

The CDC[3] recommended developing a blood lead training program applicable to all blood collection and analysis techniques. (Continued on page 8)

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(Continued form page 7)

Working with the National Laboratory Training Network and the Association of Public Health Laboratories, the program will feature separate training modules addressing screening of materials, specimen collection, patient management, specimen contamination, analytical methods, quality assurance, proficiency testing and data reporting. MDCH looks forward to participating in national dialog and continued refinement of blood lead collection recommendations from the CDC. Questions regarding testing are welcome by telephone: 517-335-8244; or FAX: 517-335-9776.

[1] Roper WL, Houk VN, Falk H, Binder S, Centers for Disease Control (CDC). *Preventing lead poisoning in young children: a statement by the Centers for Disease Control.* Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; October, 1991. [PB92-155076/HDM REPORT]

[2] Satcher D, Jackson RJ, Falk H, Hershovitz J, Centers for Disease Control and Prevention (CDC). *Screening Young Children for Lead Poisoning: Guidance for State and Local Public health Officials.* Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service*;* November, 1997.

[3] Miller DT, Jones RL, National Center for Environmental Health, Centers for Disease Control and Prevention (CDC). *Dear Colleague Letter*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service*;* February, 1999.

	CONTAMINATION DURING COLLECTION	COLLECTION DEVICE CONTAMINATION	POST COLLECTION CONTAMINATION	COMPLEX COLLECTION	ELEVATED UNSATISFACTORY SAMPLES	SPECIAL TO MAIL
VENOUS	NO	NO (if tested or certified “lead-free”)	NO	YES	NO	YES
MICRO.	YES	NO (if tested or certified “lead-free”)	NO	NO	NO	YES
FILTER PAPER	YES	YES	YES (possible)	NO	YES	NO

Comparison of collection methods for Blood Lead Testing in Children.

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